Report for 2001TN4041B: Investigation of Factors Controlling Transport of Microbial Pathogens in Saprolite Soils

- Other Publications:
  - McKay, L.D., A. Layton, M. Rietti-Shati, S. Direse, 2002, Transport of Microbial Pathogens and Pathogen Surrogates in Saprolite subsoils in east Tennessee, 12th Tennessee Water Resources Symposium, Nashville, TN, April 3-5, 2002
  - McKay, L.D., S.E. Driese, and K. Smith, In-review, Hydrogeology and pedology of saprolite formed from sedimentary rock parent material, eastern Tennessee, U.S.A.: Geoderma.
- unclassified:
  - McCarthy, J.F., L.D. McKay, and D.D. Bruner, 2002, Influence of ionic strength and Cation charge on transport of colloidal particles in fractured shale saprolite: Environ. Sci. Technol., v. 36, p. 3735-3743.
  - McKay, L.D., A.D. Harton, and G.V. Wilson, 2002, Influence of flow rate on transport of phage in a highly weathered and fractured shale: J. Environ. Quality, 31, 1095-1105.

Report Follows:

Problems and Research Objectives:

In recent years, most waterborne disease outbreaks in the US were associated with groundwater (Mathewson, 1998) and studies show that up to half of all US drinking-wells tested have evidence of fecal contamination (Macler and Merkle, 2000). A recent example of the risk to human health posed by pathogens in groundwater is a disease outbreak in Walkerton, Ontario in May 2000, that resulted in the hospitalization of dozens of people, 7 of whom died. The outbreak was attributed to fecal contamination of a water supply well in a fractured rock aquifer with *Escherichia coli* (Gillham, personal comm., Toronto Globe and Mail, 2000). Common sources of fecal contamination include septic systems, leaking sewer lines, cesspools and livestock feedlots (Macler and Merkel, 2000). Rural water supplies are particularly susceptible to contamination by pathogens because of the prevalence of septic fields and livestock, often in close proximity to the wells, springs or streams which provide drinking water to single homes or small communities. The risk of illness due to contamination by pathogens is increased because monitoring of these systems tends to be erratic.

In a recent natural gradient field tracer experiment in fractured saprolite (highly weathered rock) in Eastern Tennessee, non-pathogenic bacteriophage and bacteria-sized particles (microspheres) traveled at rates of 5 to 200 m/day over monitored distances of up to 35 m (McKay et al., 2000). This study and related laboratory studies of colloid transport (Harton, 1996; Haun, 1998; Cumbie and McKay, 1999) suggest the likelihood that pathogens may also travel rapidly in saprolite. Since saprolite mantles much of the bedrock of the southern Appalachians and the Valley and Ridge physiographic regions, it is possible that pathogens can travel down through the saprolite, into aquifers containing wells or springs used for water supply. There is a great need to develop improved methods for assessing risks related to transport of pathogens in saprolite soils and to develop a better understanding of the factors controlling their transport.

A variety of microbial pathogens can contaminate wells and springs, causing outbreaks of disease in humans. The enteric protozoa *Cryptosporidium* and *Giardia* are the most significant causes of reported waterborne disease in the US today (Rose and Yates, 1998). Both of them can originate in either animal or human fecal wastes. Previously, these protozoa were considered as microbial contaminants of surface water only. However, as 40 % of all the drinking water outbreaks associated with the enteric protozoa documented in the US in 1993 and 1994 occurred in well water sources, this original view has changed. Bacterial pathogens also commonly contaminate groundwater (Mathewson, 1998). They can be divided into 2 groups, those that cause intestinal infections, such as *Shigella*, *Salmonella*, diarrheagenic *Escherichia coli* and *Vibrio cholerae* and those that cause extra-intestinal infections such as *Legionella* and *Leptospira*. Enteric viruses include the enteroviruses (poliovirus, Coxsackie A and B viruses, echovirus), rotaviruses, Norwalk and Norwalk-like viruses (Abbaszadegan and Dowd, 1998). Enteric viruses are more resistant to environmental factors than are enteric bacteria, and they exhibit longer survival times in natural waters. Enteroviruses are also resistant to commonly used disinfectants (Pitt et al. 1994).

The first hypothesis for this study is that bacteria-sized pathogens will have greater mobility in saprolite than larger (Protozoan), or smaller (Virus) pathogens. This is what was observed in experiments using latex microspheres in saprolite (Haun, 1998; Cumbie and McKay, 1999), where the larger particles experienced greater losses due to settling and the smaller particles experienced greater losses due to attachment to fracture walls. The second hypothesis is that chemical composition of the effluent will have a major influence on pathogen attachment and retention in saprolite, with greater retention occurring in more concentrated solutions. This can help immobilize pathogens near the septic field, but may become less effective during seasonally wet periods when the effluent is rapidly diluted by infiltration.

The objectives of the study outlined in this proposal are to test these two hypotheses under controlled laboratory conditions, using undisturbed, representative samples of typical East Tennessee saprolite, and geochemical conditions typically found in or near a septic system. The experiments were carried out under saturated flow conditions, at flow rates similar to those observed in the field. Saturated conditions frequently develop in the upper saprolite during heavy rainstorms (Solomon et al., 1992; Wilson et al., 1993), and pathogen transport is expected to be greater during these periods.

The transport experiments utilized microorganisms that are representative of each of the three main types of pathogens: viruses, bacteria and protozoa. Of the various types of *Cryptosporidium*, *Cryptosporidium parvum* has been identified as the primary cause for illness in humans and domestic animals (Swiger, 1999). *Giardia* species isolated from humans are *Giardia lamblia*, *Giardia intestinalis*, and *Giardia duodenalis* (Swiger, 1999). The groundwater bacterial pathogens are *Shigella*, *Salmonella*, Diarrheagenic *Escherichia coli, Vibrio cholerae, Legionella* and *Leptospira*. Enteric viruses include the enteroviruses (poliovirus, Coxsackie A and B viruses, echovirus), rotaviruses, Norwalk and Norwalk-like viruses.

The influence of groundwater geochemistry was be evaluated by repeating the transport experiments using influent solutions that are representative of conditions both near a septic field (high ionic strength, high dissolved organic carbon, etc.), and further downgradient, where the contaminants are much more dilute. The tracer experiment will be performed separately for each type of pathogen, and then will be repeated using a mixture of all three pathogen types.

7. Methodology:

A column of interbedded shale/limestone saprolite was excavated from about 50-80 cm depth at a research site near Clinton, TN (McKay et al., in review). The sample was located in the soil-saprolite transition zone, which is a zone of relatively high hydraulic conductivity (about 2×10^{-5} m/s). This is also identified as the "stormflow zone" and is characterized by development of perched water table conditions and rapid downslope flow during periods of heavy rain .

The 25 cm diameter by 30 cm long saprolite column was set up in a permeameter in the laboratory and a series of tracer experiments were carried out using MS-2, PRD-1, *E. coli, P. fluorescens*, and a killed protozoan tracer, under different geochemical conditions. The protozoans never made it through the column, presumably due to losses from straining or settling. Breakthrough of all the bacterial and viral tracers were observed, although because of difficulties in getting quantitative measurements of some of the tracers it isn't clear whether there was actually an "optimum size" for transport as had previously been observed for experiments using microspheres. Ionic strength of the tracer solution had a strong effect with greater recovery of the microbial tracers occurring in the more dilute solutions.

8. Principle Findings and Significance:

The experimental results of the current experiments are generally consistent with our previous studies, which were based mainly on experiments using latex microspheres (Cumbie and McKay, 1999; McCarthy, McKay and Bruner, 2002) or bacteriophage (McKay, Harton and Wilson, 2002). Together, the studies demonstrate that pathogens or pathogen surrogates can be rapidly transported in typical sedimentary rock saprolite. The principal environmental controls on transport are fracture or macropore aperture, flow rate, particle size, ionic strength, valence state of the dominant cations, pH, and temperature. Each of the laboratory scale experiments addressed a different factor, usually by varying that factor while keeping the others constant. The results of the laboratory-scale tracer experiments are summarized in Table 1. They indicate that there is an optimum size for transport, of about 0.5 to 1 micron, which is about midway between the size of typical bacteria and typical viruses. This indicates that both bacteria and viruses are likely to be mobile in groundwater in saprolite. The studies also indicate that under conditions of high flow rate and low ionic strength, losses of particles due to the above mentioned factors are minimal. These conditions (high flow and low ionic strength) typically occur during periods of high precipitation, which suggests that pathogen transport will tend to be intermittent. In summary, both bacterial and viral pathogens are likely to be mobile in saprolite, and there is potential for them to be transported quickly and with little concentration loss during seasonally wet periods or during heavy rainstorms.

Table 1. Summary of laboratory-scale particle tracer experiments.

Experimental factor varied	Summary of results	References
Particle diameter (microspheres)	Optimum particle size of 0.5 to 1 micron. Electrostatic attachment dominates, with settling as a secondary factor.	Cumbie and McKay (1999)
Flow velocity (bacteriophage)	Losses due to attachment are strongly effected by flow rate, with almost no loss at high flow rates	McKay, Harton and Wilson (2002)
Groundwater chemistry (microspheres)	Particle losses are minimized at low ionic strength and in	McCarthy, McKay and Bruner (2002)

	solutions dominated by monovalent cations	
Type of microbial tracer (MS-2, PRD-1, *E. coli, P. fluorescens*, and protozoa) and groundwater chemistry.	Protozoa are completely lost to straining or settling. Losses of bacteria and viruses are strongly influenced by ionic strength.	McKay, Layton and Rietti-Shati (manuscript in-preparation)

9. Future Research and Funding:

The most significant result of the WRRIP project was that it was the first step for the principal investigators, Drs. McKay and Layton, in the field of pathogen research. Our initial theoretical research on colloid transport is now being applied to practical issues related to pathogens in waters in Tennessee. Since this project began, we have developed a major research initiative, which includes Drs. John McCarthy and Randy Gentry at UT, as well as other researchers at TDEC and USGS. Several projects have already been funded as follows:

- L. McKay and A. Layton (with a subcontract to G. Johnson at USGS), TDEC, $186,611, "Assessing the Extent of Viral Contamination and the Effectiveness of Wellhead Protection Setbacks for Wells and Springs in Fractured and/or Karst Aquifers"

- L. McKay and A. Layton, TDEC, $30,000, "Development and Testing of a Real-Time PCR Assays for the Quantification of E. coli and Host-Specific Fecal Anaerobes in Surface Waters"

We are also working on an effort to examine methods of assessing the impact of pathogens on TMDLs using Stock Creek in south Knoxville as our "research watershed". For this project we are working very closely with TDEC and City/County government and are looking at a variety of funding sources, including TDEC, TVA and local utilities.